Freshwater Toxicity Identification Evaluation

1.0 OBJECTIVE

Toxicity identification evaluations (TIEs) are used to determine the cause(s) of toxicity in freshwater samples. Laboratory tests designed to determine the toxicity of low salinity water samples typically expose *Ceriodaphnia* neonates for 96 hours, but other organisms may be used. In order to correctly interpret toxicity results, concentrations of chemical contaminants should be analyzed, as well as other water quality parameters, such as pH, dissolved oxygen, total dissolved solids, hardness, salinity, temperature, ammonia and conductivity.

In this procedure, the toxicity of the water sample tested in an initial test. If the initial test is toxic then TIE procedures are initiated. Subsamples undergo various treatments designed to remove or mitigate contaminants that might cause toxicity (US EPA 1991). Each TIE is specifically designed for the sample toxicity, organism and study objective. The TIE worksheet reflects the specific design.

2.0 EQUIPMENT

The following equipment is necessary to conduct a toxicity identification evaluation at the Marine Pollution Studies Laboratory at Granite Canyon (MPSL). The word "clean" here and throughout this procedure means that the item has been cleaned according to the MPSL glassware cleaning procedures outlined in a separate standard operating procedure (SOP).

2.1 Basic Exposures

- Procedures worksheet (very important – individually created for each TIE)
- 50-ml glass beakers or 20-mL scintillation vials
- Flasks, cylinders and pipettes of various sizes
- Environmental chamber
- Data sheets
- Gloves and appropriate safety gear (see MPSL lab safety manual)
- Analytical balance

2.3 TIE Treatments

- Refrigerated centrifuge
- Centrifuge bottles
- Pasteur pipettes
- Positive pressure pump
- Pump tubing

- Solid-phase extraction columns (C8, C18, Cation)
- Column hardware
- Reagent grade HCl and NaOH (1M) for pH adjustment
- Sodium thiosulfate
- EDTA
- Methanol
- Piperonyl butoxide

2.3 Water Quality

- Meters and probes for measuring pH, dissolved oxygen, hardness, ammonia, and conductivity
- Thermometers (glass mercury thermometer and continuously recording thermometer)
- Graduated pipettes (10-ml) and hand pipette pump for water quality sampling
- Gloves and appropriate safety gear (see MPSL lab safety manual)

2.4 Dilution Water

Type of dilution water depends on the test organism.

3.0 CONTROLS

Every treatment manipulations are performed on control water and the sample to ensure the treatment is not causing a toxic artifact.

4.0 BASELINE TEST

Prepare the appropriate volumes of each concentration.

5.0 AERATION

Bubble the appropriate amount of control water sample for one hour in beakers or flasks.

6.0 CENTRIFUGATION

Centrifuge the appropriate amount of control water sample for this treatment and for Column treatment.

7.0 EDTA

Prepare EDTA stock solution by weighing out 2.78 g of EDTA*2H2O reagent in 100 mL distilled water (yielding a concentration of 25 g/L). Use a stir bar to insure that solid EDTA has completely dissolved. Add the appropriate amount of EDTA to each sample concentration. Allow the EDTA to interact with the samples for three hours before adjusting pH back to initial pH of sample. The final concentration of EDTA is 60 mg/L.

8.0 STS

Prepare STS stock solution by weighing out 2.35 g STS*5H2O reagent in 100 mL distilled water (yielding a concentration of 15 g/L). Add the appropriate amount of EDTA to each sample concentration.

9.0 C8 COLUMN RINSATE

Use centrifuged sample. Set pump flow to 10 mL/min. Prepare tubing by pumping 25 mL distilled water followed by 25 mL MEOH. Prepare column by pumping 30 mL MEOH through column. Do not let column dry out. Pump 50 mL distilled water through column.

Pump appropriate amount of control water through column. Discard the first 20 mL and collect the remainder for testing. Re-prepare the column before pumping sample. Pump appropriate amount of centrifuged sample through column. Discard the first 20 mL and collect the remainder for testing. Run column dry and blast out extra moisture with syringe. Dilute sample to the test concentrations.

10.0 C8 COLUMN ELUATE

Elute column by removing adapter and adding 1.5 mL MEOH. Start pump at 2-mL/min. and pump column dry. Add another aliquot of 1.5 mL MEOH and pump the column dry. Collect both aliquots of eluate in a small vial. Add methanol sample to appropriate amount of control water and dilute for testing using control water that contains a similar methanol concentration.

11.0 PH SHIFT

Prepare two sets of control and each concentration. Adjust one set to pH 3 and the other set to pH 11. Allow the samples to sit for three hours, and adjust back to initial pH.

11.0 PIPERONYL BUTOXIDE

Prepare a secondary stock by adding 180 μL super stock (28 ppt, in chemical cabinet) to 100 mL distilled water. Aliquot 1 mL of secondary stock to each 100 mL of sample.

12.0 DATA HANDLING AND TEST ACCEPTABILITY

Immediately after test termination, check the data sheet to determine whether dilution water and conductivity controls have acceptable survival (mean of > 90%). If not, notify the project officer without delay. Tests with temperature, salinity, or dissolved oxygen measurements outside the specified ranges, may be considered conditionally acceptable based on the project officer's best professional judgment.

This toxicity test procedure is considered acceptable if *Ceriodaphnia* survival in controls is greater than or equal to 80%. Sixty percent of surviving females must have produced 3 broods, and surviving females must have produced an average of 15 neonates. Tests with temperature, salinity, or dissolved oxygen measurements outside the specified ranges, may be considered conditionally acceptable based on the project officer's best professional judgment. Acceptable temperatures range from 25 ± 1°C; acceptable dissolved oxygen concentration is 60-100% saturation.

13.0 REFERENCES

U.S. Environmental Protection Agency. 1991. Methods for aquatic toxicity identification evaluations. EPA 600/6-91/003. Office of Research and Development. Washington, DC.